

WHAT IS CLAIMED IS:

- 1 1. A method of inhibiting expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:
3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
5 thereby inhibiting expression of the endogenous cellular gene by at least
6 about 20%.
- 1 2. The method of claim 1, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.
- 1 3. The method of claim 2, wherein the first and second target sites are
2 adjacent.
- 1 4. The method of claim 3, wherein the first and second zinc finger
2 proteins are covalently linked.
- 1 5. The method of claim 1, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.
- 1 6. The method of claim 5, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.
- 1 7. The method of claim 2, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.
- 1 8. The method of claim 7, wherein the first and second zinc finger
2 protein are fusion proteins, each comprising at least two regulatory domains.
- 1 9. A method of inhibiting expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:
3 contacting a target site in the endogenous cellular gene with a fusion zinc
4 finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc
5 finger protein is less than about 25 nM;

1 10. The method of claim 1, wherein the cell is selected from the group
2 consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

12. The method of claim 11, wherein the cell is a human cell

1 14. The method of claim 1, wherein the endogenous cellular gene is a
2 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM, and
3 Her2/Neu.

1 16. The method of claim 1, wherein the inhibition of gene expression
2 prevents gene activation.

1 18. The method of claim 1, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.

1 19. The method of claim 1, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.

1 30. The method of claim 29, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

$\{f^{(1)}(x)\}$ $\{f^{(2)}(x)\}$ $\{f^{(3)}(x)\}$ $\{f^{(4)}(x)\}$ $\{f^{(5)}(x)\}$ $\{f^{(6)}(x)\}$ $\{f^{(7)}(x)\}$ $\{f^{(8)}(x)\}$ $\{f^{(9)}(x)\}$ $\{f^{(10)}(x)\}$ $\{f^{(11)}(x)\}$ $\{f^{(12)}(x)\}$ $\{f^{(13)}(x)\}$ $\{f^{(14)}(x)\}$ $\{f^{(15)}(x)\}$ $\{f^{(16)}(x)\}$ $\{f^{(17)}(x)\}$ $\{f^{(18)}(x)\}$ $\{f^{(19)}(x)\}$ $\{f^{(20)}(x)\}$ $\{f^{(21)}(x)\}$ $\{f^{(22)}(x)\}$ $\{f^{(23)}(x)\}$ $\{f^{(24)}(x)\}$ $\{f^{(25)}(x)\}$ $\{f^{(26)}(x)\}$ $\{f^{(27)}(x)\}$ $\{f^{(28)}(x)\}$ $\{f^{(29)}(x)\}$ $\{f^{(30)}(x)\}$ $\{f^{(31)}(x)\}$ $\{f^{(32)}(x)\}$ $\{f^{(33)}(x)\}$ $\{f^{(34)}(x)\}$ $\{f^{(35)}(x)\}$ $\{f^{(36)}(x)\}$ $\{f^{(37)}(x)\}$ $\{f^{(38)}(x)\}$ $\{f^{(39)}(x)\}$ $\{f^{(40)}(x)\}$ $\{f^{(41)}(x)\}$ $\{f^{(42)}(x)\}$ $\{f^{(43)}(x)\}$ $\{f^{(44)}(x)\}$ $\{f^{(45)}(x)\}$ $\{f^{(46)}(x)\}$ $\{f^{(47)}(x)\}$ $\{f^{(48)}(x)\}$ $\{f^{(49)}(x)\}$ $\{f^{(50)}(x)\}$ $\{f^{(51)}(x)\}$ $\{f^{(52)}(x)\}$ $\{f^{(53)}(x)\}$ $\{f^{(54)}(x)\}$ $\{f^{(55)}(x)\}$ $\{f^{(56)}(x)\}$ $\{f^{(57)}(x)\}$ $\{f^{(58)}(x)\}$ $\{f^{(59)}(x)\}$ $\{f^{(60)}(x)\}$ $\{f^{(61)}(x)\}$ $\{f^{(62)}(x)\}$ $\{f^{(63)}(x)\}$ $\{f^{(64)}(x)\}$ $\{f^{(65)}(x)\}$ $\{f^{(66)}(x)\}$ $\{f^{(67)}(x)\}$ $\{f^{(68)}(x)\}$ $\{f^{(69)}(x)\}$ $\{f^{(70)}(x)\}$ $\{f^{(71)}(x)\}$ $\{f^{(72)}(x)\}$ $\{f^{(73)}(x)\}$ $\{f^{(74)}(x)\}$ $\{f^{(75)}(x)\}$ $\{f^{(76)}(x)\}$ $\{f^{(77)}(x)\}$ $\{f^{(78)}(x)\}$ $\{f^{(79)}(x)\}$ $\{f^{(80)}(x)\}$ $\{f^{(81)}(x)\}$ $\{f^{(82)}(x)\}$ $\{f^{(83)}(x)\}$ $\{f^{(84)}(x)\}$ $\{f^{(85)}(x)\}$ $\{f^{(86)}(x)\}$ $\{f^{(87)}(x)\}$ $\{f^{(88)}(x)\}$ $\{f^{(89)}(x)\}$ $\{f^{(90)}(x)\}$ $\{f^{(91)}(x)\}$ $\{f^{(92)}(x)\}$ $\{f^{(93)}(x)\}$ $\{f^{(94)}(x)\}$ $\{f^{(95)}(x)\}$ $\{f^{(96)}(x)\}$ $\{f^{(97)}(x)\}$ $\{f^{(98)}(x)\}$ $\{f^{(99)}(x)\}$ $\{f^{(100)}(x)\}$

1 31. A method of activating expression of an endogenous cellular gene,
2 the method comprising the step of:
3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
5 thereby activating expression of the endogenous cellular gene to at least
6 about 150%.

1 32. The method of claim 31, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.

1 33. The method of claim 32, wherein the first and second target sites
2 are adjacent.

1 34. The method of claim 33, wherein the first and second zinc finger
2 proteins are covalently linked.

1 35. The method of claim 31, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.

1 36. The method of claim 35, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.

1 37. The method of claim 32, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.

1 38. The method of claim 37, wherein the first and the second zinc
2 finger protein are fusion proteins, each comprising at least two regulatory domains.

1 39. A method of activating expression of an endogenous cellular gene,
2 the method comprising the step of:
3 contacting a target site in the endogenous cellular gene with a fusion zinc
4 finger protein comprising n fingers and a regulatory domain, wherein the K_d of the zinc
5 finger protein is less than about 25 nM;
6 thereby activating expression of the endogenous cellular gene to at least
7 about 150%.

1 50. The method of claim 31, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a

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3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 51. The method of claim 50, wherein the expression vector is a viral
2 expression vector.

1 52. The method of claim 50, wherein the expression vector is a
2 retroviral expression vector, an adenoviral vector, a DNA plasmid vector, or an AAV
3 expression vector.

1 53. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 54. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 55. The method of claim 31, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 56. The method of claim 31, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 57. The method of claim 31, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 58. The method of claim 31, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 59. The method of claim 31, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 60. The method of claim 59, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

1 61. A method of modulating expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:

- 1 72. The method of claim 71, wherein the cell is a human cell.
- 1 73. The method of claim 61, wherein the endogenous cellular gene is a
2 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM,
3 Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.
- 1 74. The method of claim 61, wherein the endogenous cellular gene is
2 VEGF.
- 1 75. The method of claim 65 or 67, wherein the regulatory domain is
2 selected from the group consisting of a transcriptional repressor, a transcriptional
3 activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone
4 deacetylase.
- 1 76. The method of claim 61, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.
- 1 77. The method of claim 61, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.
- 1 78. The method of claim 61, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a
3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.
- 1 79. The method of claim 78, wherein the expression vector is a viral
2 expression vector.
- 1 80. The method of claim 78, wherein the expression vector is a
2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression
3 vector, or an AAV expression vector.

1 81. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 82. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 83. The method of claim 61, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 84. The method of claim 61, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 85. The method of claim 61, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 86. The method of claim 61, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 87. The method of claim 61, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 88. The method of claim 88, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

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